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Review

Cytisine for the treatment of nicotine addiction: from a molecule to therapeutic efficacy

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Abstract:
Cytisine, a natural plant alkaloid, has been marketed in Central and Eastern Europe for over 40 years for the clinical management of smoking cessation. Despite the fact that cytisine has been used by millions of smokers, its characteristics have not been reviewed in scientific literature in English, and presently existing clinical studies on its effectiveness and safety are insufficient to warrant licensing by modern standards. Understanding of the mechanism of cytisine action as a smoking cessation aid provides a necessary basis for conducting clinical trials to confirm its efficacy as an optimal antismoking therapy. Hereafter, we present a review of current knowledge about the pharmacokinetics, pharmacodynamics, toxicity, therapeutic efficacy and safety of cytisine, and about its derivatives that are under development. Recent pharmacological research has elucidated that the drug is a low efficacy partial agonist of $\alpha 4\beta 2$ nicotinic acetylcholine receptors, which are believed to be central to the effect of nicotine (NIC) on the reward pathway. The drug reduces the effects of NIC on dopamine release in the mesolimbic system when given alone, while simultaneously attenuating NIC withdrawal symptoms that accompany cessation attempts. Clinical studies on cytisine as a smoking cessation aid have demonstrated that the drug is effective and safe. Our recent uncontrolled trial has shown that a 12-month carbon monoxide-verified continuous abstinence rate following a standard course of treatment with cytisine with minimal behavioral support is similar (13.8%; N = 436) to that observed following treatment with NIC replacement therapy. Since cytisine exhibits a desirable pharmacological profile which makes it an attractive smoking cessation drug, it should be advanced to randomized clinical trials. However, more detailed preclinical studies on its pharmacokinetics and safety profile are required.

Key words:
cytisine, nicotine addiction, smoking cessation, nicotine, dopamine, tobacco dependence, varenicline

Abbreviations: ACh – acetylcholine, CEE – Central and Eastern Europe, CNS – central nervous system, CO – carbon monoxide, CYP – cytochrome P450, DA – dopamine, *ip* – intraperitoneal, *iv* – intravenous, nAChRs – nicotinic acetylcholine receptors, NE – norepinephrine, NIC – nicotine, NRT – nicotine replacement therapy, *po* – per os, *sc* – subcutaneous

Introduction

Tobacco smoking is one of the main threats to human health and is the biggest single factor of premature mortality in Poland and worldwide. It is estimated that in XXI century 1 million smokers will die because of their smoking [115]. Despite attempts to control tobacco use covering a broad spectrum of interventions, breaking the nicotine (NIC) habit remains difficult.

The best results in the treatment of NIC addiction are achieved when a combination of pharmacotherapy and non-pharmacological treatments, including additional behavioral support, is applied. The current most effective pharmacological tools are nicotine replacement therapy (NRT) and bupropion [101], and in some countries recently approved varenicline. NRT

increases 12-month continuous abstinence rate in smokers about 1.5 to 2 times in comparison to placebo [134]. Bupropion doubles the chances of success of quit attempts [64]. Despite their advantages, current pharmacotherapies are too expensive for many smokers, especially in developing countries, and are not widely disseminated to the general population of smokers. It is supposed that new medicines, like varenicline, rimonabant, and NIC vaccines will be expensive and unaffordable for many smokers. Thus, there is an urgent need for identification and evaluation of other forms of pharmacotherapy which would be effective, safe and less expensive for health care systems and smokers. Modern development of such therapies should be based on the conclusions from preclinical studies explaining neurobiological mechanism(s) underlying NIC addiction and identifying targets for pharmacological treatments. Data derived from these studies suggest that an optimal antismoking agent should mimic the behavioral and biochemical effects of NIC but should be devoid of addictive liability or positive reward [46].

These guidelines of mimicking the biochemical effects of NIC yet lacking addictive or positive rewarding properties led us to focus on cytisine, an alkaloid of plant origin marketed for over 40 years in Central and Eastern Europe (CEE) [146, 148, 149, 159, 160]. In addition, our recent trial confirming its efficacy and safety and potential low cost of the therapy encouraged us to propose cytisine as an attractive drug for smoking cessation that should receive wider awareness.

Background

Origin of cytisine

Cytisine is a quinolizidine alkaloid originating from seeds and many other parts of plants of the *Leguminosae* (*Fabaceae*) family, including *Laburnum, Sophora, Baptisia* and *Ulex* spp. [69, 93]. The greatest amount of the alkaloid is found in the seeds of the common garden decorative plant *Laburnum anagyroides* (*Cytisus laburnum*; Golden Rain accacia*)* (about 1–5%). In 1912, Dale and Laidlaw [37] have described cytisine to be the toxic component of this plant.

History of the use in medicine

The extracts from the *Laburnum* seeds and flowers have been used in traditional medicine for hundreds of years. However, a historical clock for cytisine started thousands years ago in America where Indians have consumed the seeds for their emetic and purgative effects during rites and magical practices [42, 94, 156]. In Europe, traditional medicine has recommended alcoholic extracts containing cytisine for constipation, migraine, insomnia, cough and neuralgias. About 100 years ago, cytisine was used as an antiasthmatic agent and an insecticide. During the Second World War the leaves of *Laburnum anagyroides* were used as a tobacco substitute [132]. There are also reports indicating that cytisine or cytisine-containing plants have been administered as a diuretic in Western Europe [82], an analeptic in the former Soviet Union [39, 158] as well as an agent replacing NIC in smokers making a quit attempt in CEE [55, 86].

Cytisine as a smoking cessation aid has been used since the 1960s in Bulgaria. The first clinical study using cytisine for smoking cessation was carried out by Stoyanov and Yanachkova in 1965 [141]. In the next 10 years, other pharmacological and clinical studies in Bulgaria, Poland, Russia, East and West Germany were performed, demonstrating good efficacy and safety of the drug [11–14, 58, 76, 91, 112, 113, 129, 130, 141, 142]. Since the results of those studies were promising, cytisine was developed, and has been manufactured and marketed from 1964 as Tabex® (Sopharma, Bulgaria), and has been widely distributed in CEE.

Poland is one of the countries where cytisine has been widely used. During the last 40 years probably hundreds of thousands of smokers have been using cytisine while quitting. An important factor that decides about a wide use of the drug is its low cost. The cost of a full course of the treatment in Poland is approximately the equivalent of 10 $ or 8.9 € (for more information see: www.bpg.bg/tabex); thus, much less than the cost of NRT or bupropion. However, despite its 40+ years on the market, there have been no reports of clinical observations or studies published in scientific literature in English. Consequently, cytisine is unknown outside of CEE. At least to our knowledge, cytisine has not been used for therapeutic purposes in Western countries. Very recently, a review of the efficacy data confirming the therapeutic potential of cytisine has been published in English [44].

Cytisine appears to be the oldest medication used for smoking cessation. After many years of oblivion, cytisine is presently a medication interesting to chemists, pharmacologists and clinicians because of its potential to be an inexpensive, safe and effective medication for smoking cessation. Recent clinical data have demonstrated the effectiveness of cytisine for smoking cessation. Cytisine also has intrigued researchers because of the clinical success of one of its synthetic derivatives – varenicline.

Chemistry

Although cytisine was isolated already in 1863 by Husemann and Marme, its chemical structure was described only in the 1930s [66–68].

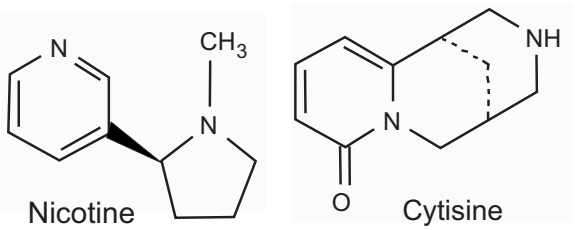

Fig. 1. Chemical structures of cytisine and nicotine. Reprinted from [30]

Chemically, cytisine is (1R-cis)-1,2,3,4,5,6-hexahydro-1,5-methano-8*H*-pyrido[1,2-*a*][1,5]diazocin-8-one ($C_{11}H_{14}ON_2$). The structural formula is depicted in Figure 1. X-ray crystal structure analysis indicates that its chemical structure closely resembles that of NIC [9]. The quasi-aromatic ring in cytisine and the pyridine ring of NIC are associated in a similar way in relationship to the nitrogen atom in the bispidine ring and the nitrogen atom in the pyrrolidine ring, respectively [9].

Cytisine crystallizes as big colorless crystals, easily soluble in water, chloroform and ethanol, less soluble in benzene, ethyl acetate and acetone, and insoluble in ether [30].

Cytisine is a compound with a relatively rigid conformation. The rigidity of the molecule makes it an attractive template for structure-activity studies. In recent years, the studies on the structural modification of cytisine have led to the development of novel compounds of potential therapeutic interest [1, 18, 19, 21, 31, 34, 50]. One of such compounds, varenicline has recently been approved by the US Food and Drug Administration for the treatment of NIC addiction [31–33, 54, 73, 102, 145].

Pharmacology

Pharmacokinetics: preclinical animal studies

The available data on the disposition kinetics and metabolism of cytisine derive from animal studies. Pharmacokinetics was investigated after a single per os (*po*) or intravenous (*iv*) administration of the drug at a dose of 2 mg/kg in mice [79], and after a single *po* (5 mg/kg) or *iv* (1 mg/kg) administration in rabbits [143]. The results of the latter study are shown in Table 1.

Absorption

Cytisine given *po* was readily absorbed in the gastrointestinal tract [81]. In mice, approximately 42% of the drug administered *po* was absorbed with the time to reach maximum blood concentration of 120 min [79]. Oral bioavailability in rabbits was 34% and

Tab. 1. Pharmacokinetic parameters of cytisine after oral or intravenous administration in rabbits

Route of administration	Bioavailability %	C_{max} µg/l	T_{max} min	Volume of distribution l/kg	Elimination half-life min	Renal clearance ml/min
Oral (5 mg/kg; N = 8)	34	388.9	35	6.2	52	167.2
Intravenous (1 mg/kg; N = 10)	–	–	–	1	37	43

Adapted from [143] with permission of Sopharma

maximum plasma concentration was observed after 35 min [143].

Transdermal application in rabbits resulted in a steady state that was reached in two phases [127]. The first phase lasted 24 h, and the second phase continued through the next 3 days. In the first phase, drug absorption capacity and concentration in blood were twice higher than in the second phase [127].

Distribution

After a single *po* or *iv* administration in mice, the highest concentration of cytisine was found in the liver, adrenal glands and kidneys. Following *iv* administration, the highest concentration of the drug in the bile was 200 times that in the plasma [79].

The ability of a drug to cause dependence is related to its ability to penetrate into the central nervous system (CNS). The lipophilic characteristics of a drug are most frequently used to indicate the probable penetration of the drug through the blood-brain barrier. The partition coefficient among organic solvents and water for cytisine is lower than for NIC and is expressed as the $\log_w$ of approximately 0.21 for cytisine and 1.24 for NIC at pH = 7.4 [122]. When cytisine was administered to rats at a dose of 1 mg/kg, its concentration in plasma was 516 ng/ml, and in the brain 145 ng/ml. Thus, the brain concentration was not more than 30% of the plasma concentration. For comparison, administration of the 0.1 mg/kg dose of NIC resulted in a mean plasma NIC concentration of 62 ng/ml and the concentration in the brain was 65% of that in plasma. These data show that cytisine weakly penetrates the blood-brain barrier as compared to NIC [122, 124]. Therefore, trials of drug modification to improve the penetration of the blood-brain barrier have been performed [18].

Metabolism and elimination

The half-life assessed after *iv* single dose of cytisine was 200 min in mice and was longer than the half-life of NIC [79, 147]. Approximately 32% of the dose could be recovered from the urine over 24 h and 3% from feces over 6 h**.** After *po* application, 18% of the applied dose was excreted within 24 h in the urine [79]. In rabbits, the elimination half-life was 37 and 52 min after *iv* or *po* administration, respectively [143].

In contrast to NIC, cytisine undergoes minimal metabolism and 90–95% of its dose is excreted unchanged in the urine [147]. The clearance rates after *po* and *iv* application in rabbits were 167 and 43 ml/min, respectively [143].

So far, the pharmacokinetics of the drug in humans has not been established. It is critical considering regulatory requirements, that the pharmacokinetics and pharmacodynamics in humans is established. In addition, pharmacokinetics is essential in special patient populations, e.g. in patients with renal insufficiency, geriatric and pediatric patients. Also, sex- or smoking status-related differences in kinetics should be defined. Studies of drug metabolism exploring the potential influence on hepatic P450 enzymes should also be performed.

Pharmacodynamics

Receptor binding

The evidence that NIC is responsible for the dependence on tobacco has been reviewed many times [15]. It is commonly accepted that NIC addiction results, at least partially, from its interaction with neuronal nicotinic acetylcholine receptors (nAChRs). NAChRs are ligand-gated cation channels widely expressed in the brain, autonomic ganglia, adrenal medulla and neuromuscular junctions, and on numerous other cell types [126]. Postsynaptic nAChRs exert a stimulatory effect on neurons, presynaptic nAChRs modulate while synaptic transmission by the release of many neurotransmitters [36, 71].

NAChRs types are pentamers formed from different combinations of genetically distinct subunits $\alpha_{(1–10)}$, $\beta_{(1–4)}$, γ, δ, and ε [74, 88]. Although a large number of neuronal subtypes have been identified, $\alpha4\beta2^*$, $\alpha3\beta4$, and $\alpha7$ predominate in the brain [88]. So far, the studies on cytisine have focused on targeting these three most prevalent subtypes of nAChRs.

The presynaptic $\alpha4\beta2^*$ (where * denotes the possible inclusion of an additional, unspecified subunits [88]) nAChRs modulate dopamine (DA) release and overflow from dopaminergic terminals in the mesolimbic system, which are believed to be central to the effect of NIC on the reward pathway [22, 125]. The development of new subtype-selective $\alpha4\beta2^*$ ligands has been shown to be successful for treating NIC addiction [54, 73, 145].

Affinity. A substantial amount of information on pharmacodynamics of cytisine has come from studies on neuroblastoma cell lines which naturally express

nAChRs and from rat or human recombinant nAChRs expressed in *Xenopus laevis* oocytes. *In vitro* binding studies showed that cytisine had a million-fold higher selectivity for nAChRs over muscarinic acetylcholine receptors [3]. In the rat whole-brain membrane preparations, cytisine exhibited higher affinity for nAChRs (however, no specific subunit distinction was determined) than NIC [3]. On the other hand, there is a study in which no significant differences between cytisine or NIC were demonstrated [107]. In rats, the affinity of cytisine for nAChRs was the highest in the thalamus, striatum and cortex [107]. In human brain membrane preparations, the highest density of cytisine binding sites was shown in the thalamus [59]. An autoradiographic study by Hall et al. [60] in rhesus monkey demonstrated the localization of binding sites for [(3)H]cytisine and [(3)H]NIC in layer IV of some cortical areas, with mostly the thalamic nuclei, and presubiculum displaying high levels of labeling for cytisine. Moderate levels of binding were demonstrated in the subiculum, septum, and mesencephalon, and low levels were demonstrated in layers I, II and VI of the cortex, dentate gyrus, and amygdala [60].

Cytisine differs in the affinity for different subtypes of nAChRs. The alkaloid binds with high affinity predominantly to α4β2* subtype [31, 50, 59, 63, 98, 107, 110, 135, 137, 161]. The experiments comparing the binding of cytisine and nicotine to α4β2* nAChRs demonstrated that the affinity of cytisine was about 7-fold higher than that of NIC [31, 65]. The autoradiographic study of rat brains demonstrated that cytisine bound to α4β2* nAChRs at nanomolar concentration (K_i = 0.45 nM, [107]; K_i = 2.4 nM, [19]). These data are similar to results of experiments with rat receptors expressed in *Xenopus* oocytes (K_i = 1.03 nM, [110]; K_i = 0.17 nM, [31]) or cell lines (K_i = 0.12 nM, [50]) and data from the studies using recombinant human receptors expressed in *Xenopus* oocytes or cell lines (K_i = 1.07 nM, [63]; K_i = 1.2 nM, [135]).

It has been reported that the affinity of α4β2* nAChRs for many ligands differs significantly depending on whether receptors are examined in intact cells (*Xenopus* oocytes) or in cell homogenates [47]. Zhang and Steinbach [161] compared binding of cytisine and NIC to specific sites in homogenates prepared from HEK 293 cells which stably express human neuronal α4β2* receptors or to surface receptors on intact cells. They found that cytisine bound to surface receptors in intact cells, and showed an insignificant difference in affinity between these receptors and the receptors in cell homogenates. The binding of NIC to receptors on intact cells was about 4-fold higher than binding of cytisine. When NIC and cytisine competed for binding sites of intact cells, NIC fully blocked cytisine binding but cytisine only partially inhibited NIC binding [161].

Cytisine binds to α3β2* nAChRs with relatively low affinity [108]. The potency of cytisine was lower than those of epibatidine and NIC, and similar to the potency of acetylcholine (ACh)[25].

The α3β4* nAChRs are present in autonomic ganglia where they mediate synaptic transmission, and represent a minority population of NIC receptors in the brain. As demonstrated in cultured cells or *Xenopus* oocytes, cytisine bound and activated α3β4* receptors at low micromolar concentrations (K_i = 840 nM, [31]; K_i = 220 nM, [50]) with an affinity slightly lower than that of NIC. The affinity of cytisine for α3β4* receptors was 11-fold and 47-fold lower than the affinity for α2β4* and α4β4* receptors, respectively [110].

Homomeric α7 receptors are relatively widespread in the brain, have the highest Ca^{2+} permeability and the fastest desensitization kinetics of the nAChRS, and are involved in glutamate release in the hippocampus [118, 133]. According to the study on recombinant human receptors by Chavez-Noriega et al. [24], cytisine is a full agonist of α7 nAChRs, displaying an affinity which is > 24000-fold and 5-fold lower than the affinities for α4β2* and α3β4* subtypes, respectively [31]. The affinity for α7 receptors was at low micromolar concentrations with the values of K_i ranging from 4.2 to 8.4 μM [31, 63, 135]. In comparative binding assays reported by Imming et al. [65], the affinity of cytisine for α7 receptors was 2-fold lower than that of NIC.

The rank order of cytisine affinity derived from experiments on receptors expressed in *Xenopus* oocytes or cell cultures is the following: α4β2* > α4β4* > α2β4* > α3β2* > α3β4* > α7 [31, 110].

Cytisine exhibited very limited binding to receptors for cholecystokinin (A), histamine (H_3), kainic acid, N-methyl-D-aspartic acid (NMDA), phencyclidine, serotonin (5-HT_3), thyrotropin-releasing hormone (TRH), vasoactive intestinal peptide (VIP), and had no affinity for receptors for adenosine (A_1 and A_2), angiotensin II, galanin, insulin, interleukin 1α, leukotriene B_4, neurokinin 1, neuropeptide Y, platelet-activating factor, serotonin (5-HT_{1A}), tromboxane (A_2), tumor necrosis factor as well as muscarinic (M_1 and M_2) and sigma receptors [19].

Efficacy. The functional effects of cytisine are markedly determined by its agonist efficacy at each nicotinic ACh receptor. The first electrophysiological studies on the alkaloid's agonist/antagonist activity were performed by Luetje and Patrick [87] as well as Papke and Heinemann [108]. In their experiments, the responses of α4β2*- and α3β2*-injected oocytes to the application of 1 mM cytisine amounted only to 15% and 2.5% of the responses to 1 mM ACh, respectively. The coadministration of cytisine and ACh resulted in the reduction of the responses to ACh. Thus, they suggested that cytisine was a partial agonist of β_2-containing nAChRs. The study by Coe et al. [31] confirmed this suggestion. Using 10 μM concentration of cytisine and measuring the current evoked at α4β2* nAChRs, Coe et al. [31] estimated agonistic activity of the drug at 56% of the response to 10 μM of NIC. When both agents were co-applied, cytisine partially blocked (30% inhibition) effect of NIC. So, it clearly indicated the partial antagonistic profile of cytisine. In the comparative studies, the efficacy of the drug was similar at both rat and human α4β2* nAChRs [21, 24, 109].

Cytisine shows a wide variation in functional efficacy at different subtypes of nAChRs. Efficacy at α4β2* was much lower than at various β4*-containing and α7 receptors [21, 63, 109, 135].

Taken together, the above-described findings from *in vitro* and *in vivo* assays indicate that cytisine has high affinity for rat, rhesus monkey and human cerebral nAChRs, displaying selectivity especially to α4β2* subtype. The affinity of cytisine for α4β2* receptors seems to be superior to that of NIC and many other nAChRs agonists. The drug has negligible affinity for many neurotransmitter and hormone receptors. Cytisine displays low efficacy at α4β2* and other β2*-containing receptors. In other words, cytisine is a low-efficacy partial α4β2* agonist and a full agonist at α7 and α3β4* nAChRs.

Pharmacological action

Based on our understanding of cytisine pharmacology, it appears to be of potential therapeutic utility in aiding smoking cessation and possibly in treating other diseases. The first data on pharmacological action of cytisine were reported at the beginning of 20th century [37, 157]. Paskov and Dobrev [111], continuing the investigations begun in the former Soviet Union, compared the pharmacological action of cytisine and NIC. Ten years later, Daleva and Sheykova [38] published an additional study on pharmacological properties of cytisine and its derivatives.

The alkaloid exerts central and peripheral effects that are similar to the effects of NIC. The differences in effects between NIC and cytisine are related to the concentration of the drug rather than to sites of its action or response.

Central nervous system effects: clinical targets for cytisine

The central effects of cytisine resemble the effects of NIC at the nAChRs. Its central activity results from the stimulation of nAChRs resulting in the modulation of the release of neurotransmitters, such as DA and norepinephrine (NE).

Mechanism of antismoking action. It is widely accepted that the development of NIC addiction and the neurobiological mechanisms explaining NIC reinforcement, withdrawal syndrome and relapse depend on its action as an agonist on α4β2* nAChRs. The α4β2* nAChRs stimulation results in molecular changes in the nucleus accumbens and prefrontal cortex entailing dopaminergic activation [40, 41, 46]. The absence of striatal DA release in NIC-treated β2 subunit knockout mice indicates that α4β2* nAChRs play a key role in the DA-releasing effects of NIC [116]. An increase in the DA level is responsible for pharmacological reward whereas lower DA levels are associated with the withdrawal symptoms experienced by smokers during cessation attempts (Fig. 2).

Modulation of [^{3}H]DA release by cytisine *via* β2*-containing nAChRs was shown in the studies by Grady et al. [57] and Abin-Carriquiry et al. [1]. The latter study used native rat nicotinic receptors and showed that cytisine elicited the [^{3}H]DA release from striatal slices with maximum release that was approximately 50% of that seen with NIC.

The partial agonist effect of cytisine at α4β2* nAChRs was also demonstrated *in vivo* by measurement of the DA turnover (a measure of the utilization and synthesis of DA) in the nucleus accumbens of conscious Sprague-Dawley rats [31]. When given alone *sc* at a dose of 5.6 mg/kg, the drug increased the DA turnover with an efficacy amounting to 40% of the maximal NIC effect. In animals treated concurrently with NIC, cytisine inhibited the NIC response by 36% [31]. In the comparative study, the effects of

cytisine on central dopaminergic response were similar to the effects of its derivative, varenicline that increased the DA turnover with an efficacy 32% of the maximal NIC response and inhibited NIC's effect by 66% [31].

In conclusion, *in vitro* and *in vivo* results suggest that in NIC addiction, cytisine would moderately increase the DA level in the mesolimbic system, attenuating the withdrawal symptoms, and on the other hand, it should minimize the addictive effects of NIC by decreasing the DA level (Fig. 2). The lower efficacy of cytisine in causing DA release compared with NIC suggests that cytisine should be significantly less addictive. Thus, from therapeutic point of view, cytisine shows a pharmacological profile which makes it an attractive smoking cessation drug.

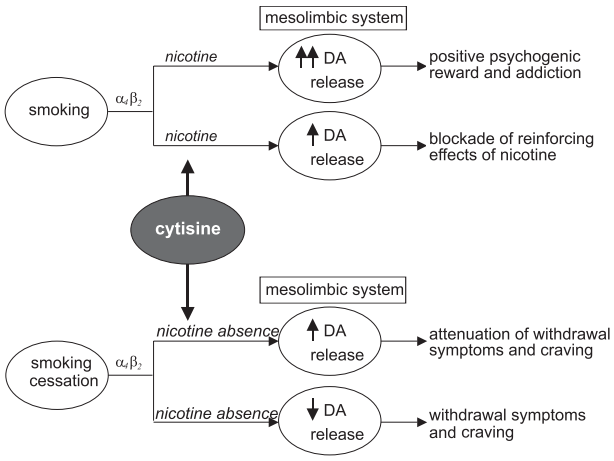

Fig. 2. Mechanism of antismoking effect of cytisine. DA – dopamine

Other central nervous system effects. Apart from its action on dopaminergic neurons in the mesolimbic system, cytisine modulates the release of NE. The NE release is governed by β4*-containing receptors [89]. The compound elicited [^{3}H]NE release from hippocampal preparations in a concentration-dependent manner with a potency approximately 50-fold lower than in the [^{3}H]DA release assay [1]. Both the potency and efficacy of cytisine in the modulation of the NE release were comparable with those of NIC [29]. The role of cytisine in the release of other neurotransmitters has not been fully determined. It is only known that cytisine appears to have no effect on the release of serotonin [119].

As mentioned above, cytisine exerts NIC-like pharmacological effects but its effects are weaker than those of NIC. Besides the mechanisms mentioned above, weaker effects of cytisine are possibly due to its poorer penetration into the CNS because of a weaker lipophilic profile and low partition coefficient between organic solvent and water [122, 124]. Cytisine's lower efficacy as compared to NIC in resolution of withdrawal symptoms is probably related to pharmacokinetic effects in addition to pharmacodynamic effects. Cytisine administered to rats at a moderate dose (1 mg/kg) achieves a concentration in the brain, which should induce similar effects as those elicited by NIC. However, this does not occur. The induction of a similar response to NIC only occurs if the dose was adjusted for the known difference in the blood-brain barrier permeability and the difference in the receptor affinity.

Locomotor activity. The systemic application of NIC or other agonists of nAChRs can increase the locomotor activity and this effect is more prominent with a prolonged exposure [28, 80, 100, 121, 139]. The application of NIC into the peripheral nervous system of rats resulted in a decrease in their kinetic activity during the first 20 min. After 20 min, the kinetic activity increased. In rats chronically exposed to NIC, cytisine caused neither a decrease in kinetic activity in the first 20 min nor an increase in kinetic activity [139]. It is noteworthy that in another behavioral model based upon locomotor activity, cytisine was more potent than NIC, when it was administered by direct injection into the central tegmental area of the brain [100, 121]. The locomotor-activating effects associated with the tegmental area injections of cytisine seem to be mediated by the mesolimbic DA system [100].

Drug discrimination. Drug discrimination is a method for assessing the behavioral actions of drugs. NIC readily acts as a potent discriminative stimulus [117]. Reavill et al. [122] compared the discriminative stimulus effects of cytisine with those of NIC. They examined rats' behavior and their reaction to transdermal application of NIC or cytisine. Animals were positively rewarded with food after administration of NIC. Cytisine-appropriate response rate was 65%, while corresponding value for NIC was 95%. There was no correlation between increasing cytisine dose and the number of proper answers. Cytisine applied together with NIC did not impair significantly the reaction to NIC: rats' behavior after applying only NIC

or after applying cytisine and NIC together did not differ. In another study, in rats trained to discriminate cytisine from saline, NIC produced full dose-dependent generalization of up to 93%. In rats trained to discriminate NIC, cytisine also increased drug-appropriate responses; however, this effect was of smaller magnitude (54%). These results showed that cytisine, like NIC could serve as a robust discriminative stimulus, but it was much less potent than NIC in the behavioral studies. There are some differences between both drugs, like the asymmetrical cross-generalizations and differences in susceptibility to antagonism by mecamylamine. These studies confirm that cytisine only partially activates nicotine receptors, and does not provide equal CNS response [23].

Analgesia. The role of NIC and other nAChRs agonists in analgesia has been well documented [61]. In the hot-plate test, cytisine exhibited an antinociceptive effect, which was weaker than the effect of epibatidine, but more potent than the effect of NIC [120, 131].

Cognitive functions. Epidemiological studies indicate a negative correlation between smoking and an incidence of Parkinson's disease and, to a lesser extent, Alzheimer's disease [52]. NIC and some its analogues were shown to enhance cognitive function and are considered as a potential therapy for patients with Alzheimer's disease [70]. The question to be resolved by future research is whether cytisine can imitate NIC and would offer therapeutic benefits in patients with these diseases. Animal studies showed that cytisine, similarly to NIC, facilitated retention of avoidance training and improved memory and learning [20, 83, 84]. The beneficial action of cytisine on cognitive processes was blocked by flupentixol, an antagonist of DA D_1/D_2 receptors. It suggests that such effects of cytisine involve dopaminergic neurotransmission [20].

Neuroprotection. An increase in DA turnover is believed to compensate for impaired transmission in neurodegenerative processes, for example in Parkinson's disease. NIC facilitates the DA release from neurons in the nigrostriatal pathway, the area that is depleted of DA-containing neurons in Parkinson's disease [56]. Therefore, NIC is being investigated as a treatment for Parkinson's disease. Several models are used to mimic the neuropathology of Parkinson's disease. One model uses a neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) that causes degeneration of nigrostriatal dopaminergic neurons, decreases the DA concentration in the striatum and produces parkinsonian symptoms. *In vivo*, cytisine partially prevented the MPTP-induced reduction of the striatal DA concentration and the increase in the DA turnover [48]. Some investigators reported that the neuroprotective effects of cytisine resulted from its interaction with $\alpha 7$ subtype and, maybe, $\beta 2$*-containing subtypes of nAChRs [72, 138]. On the basis of suggestions that an increased iron release from ferritin is involved in the pathogenesis of Parkinson's disease [16], Ferger et al. [48] proposed an interesting hypothesis that cytisine might form complexes with iron and may thus lead to a decrease in hydroxyl radical production.

Therefore, the beneficial effects on cognitive function and neuroprotective properties make cytisine a possible therapeutic tool for therapy Parkinson's and Alzheimer's disease but further detailed studies are required. A few studies available to date suggest that the alkaloid might be a promising substance for studying neuroprotective effects on nigrostriatal dopaminergic neurons.

Mood. Many smokers report that smoking helps them relieve depression, and some smokers become depressed after smoking cessation. Of interest was the observation that cytisine exhibited antidepressive activity in rats by overcoming immobility in the Porsolt's test [6]. Neurochemical effects of cytisine resemble effects of some antidepressant drugs, so it is reasonable to consider cytisine as a potential therapy in smokers with depression and other affective disorders.

Peripheral effects

Systemic administration of cytisine affects the autonomic ganglia, the adrenal medulla, and carotid sinus *via* nAChRs localized in these tissues. Usually, peripheral effects are manifested after application of the drug at doses that are 1/4 to 2/3 lower than the doses of NIC required to cause the same effect [10].

Cytisine can have both excitatory and inhibitory effects in autonomic ganglia; however, the stimulating effect is more potent than its blocking effect [157]. The study of Barlow and McLeod [10] showed that cytisine stimulated cat superior cervical ganglion at concentrations 25% lower, and blocked the ganglion at concentrations 17% higher than respective concentrations of NIC causing the same effects. The stimulation of sympathetic ganglia results in an increase in blood pressure. The elevation of blood pressure was over 2-fold greater than after administration of NIC.

Also, the effects of cytisine on contraction of the guinea and rat pig ileum, the frog rectus as well as on block of the rat diaphragm were also more potent than those of NIC [10, 111, 114]. The stimulant effect on guinea pig ileum was antagonized by hexamethonium [8].

Cytisine at high doses caused desensitization of ganglionic and/or channel blockade of nAChRs [24]. As a consequence, a decrease in blood pressure and heart rate, an increase in cardiac output as well as an increase in respiration rate was observed. Animal studies showed that cytisine was 1.4 times more potent than NIC in decreasing blood pressure but was 1/4 to 1/8 as potent as NIC in decreasing heart rate, respiration rate and enhancing minute volume. Cytisine's ability to affect tidal volume was weaker than that of NIC [136].

Apart from its action on the autonomic ganglia, cytisine stimulates, in a Ca^{2+}-dependent manner, chromaffin cells in the adrenal medulla. The affinity of the alkaloid for different subtypes of nAChRs in the adrenal medulla was similar to the affinity for the ganglionic receptors [17]. Cytisine was somewhat less efficacious than NIC in adrenal chromafin cells [153]. A consequence of the activation of adrenal nAChRs is an enhanced release of catecholamines, which elevate blood pressure and blood glucose level. It is well known that stopping smoking leads to a decrease in blood pressure and blood glucose values. Cytisine, by mimicking the effects of NIC, can restore, at least partially, normal values of blood pressure and prevent the reduction of the blood glucose level and in this way it can attenuate some symptoms of NIC withdrawal.

Cytisine at low doses stimulates the respiratory center, mainly by the activation of nAChRs in carotid sinus; however, it has been suggested that cytisine can also exert a direct action on the respiratory center [111]. In anesthetized rats, the stimulating action of cytisine was less potent than that of NIC. In order to reach the same response, the dose of cytisine must be 3-fold higher than that of NIC [10].

Toxicity

Data demonstrating the toxicity of cytisine derive mainly from the studies in rodents [4, 5, 111], dogs [4, 85], cats [157] and horses [62, 123]. Moreover, several cases of accidental poisoning with *Laburnum* seeds and a case of an intentional overdose of cytisine (Tabex®) in humans have been reported [123, 142].

Animal toxicity

Acute and chronic toxicity

The therapeutic index of cytisine is wide [111]. In the acute experiment, the LD_{50} values (i.e. doses of the drug causing death in 50% of animals) obtained after *iv*, *sc*, and *po* administration of the drug were 2.3, 13, and 13 mg/kg for male, and 3.1, 13, and 29 mg/kg for female mice, respectively. In rats (of both sexes), the LD_{50}s were 9, 11, and 38 mg/kg after *ip*, *sc*, and *po* administration, respectively. When cytisine was injected *sc* in dogs, the LD_{50} value was 4 mg/kg [4].

The most frequent symptoms of toxicity in animals are from the gastrointestinal tract. The first symptom is almost always vomiting. The onset of symptoms is quick, with vomiting and other gastrointestinal disturbances appearing 45 min to 4 h after administration [4, 75, 77]. The effects of toxic doses include initially weakness, dizziness, impairment of motor coordination, atactic gait, slow movements, muscular facial twitches, increased reflex sensitivity, mydriasis, rapid, shallow breathing, occasional urinary retention, and ultimately, tonic-clonic convulsions followed by collapse and coma with respiration cessation, mainly caused by paralysis of respiratory muscles [4, 75, 77, 111, 157]. Thus, these symptoms are the same as the symptoms of NIC overdose.

Organ toxicity

Hepatotoxicity in vitro. The data about toxicity of cytisine for individual organs are fragmentary. Hepatotoxicity has been studied the most. A recent *in vitro* study assessed cellular function by determination of hepatocyte viability and the amount of reduced glutathione, which characterized the possible toxic hepatic metabolism of xenobiotics [143]. Cellular viability of hepatocytes treated with cytisine at 5 nM was reduced by 12% *vs*. untreated controls while NIC lowered it by 35% ($p < 0.001$). Cytisine (5 nM) decreased the level of reduced glutathione by 17% ($p < 0.01$) while NIC caused a 41% ($p < 0.001$) reduction in comparison to control. The effect of both compounds was dose-related. Taken together, the results indicated that cytisine exhibited lower cytotoxicity, more weakly affecting hepatocyte viability and re-

duced glutathione levels than NIC at equivalent concentrations (5 nM–250 μM).

The same study demonstrated higher toxicity of cytisine compared to NIC in the assay of malondialdehyde, a product of lipid peroxidation, which gives information on the possible cytotoxicity associated with the formation of free radicals and unlocking of lipid peroxidation processes. A possible explanation of higher toxic effects of cytisine in the malondialdehyde test could be characteristic of cytisine which, in contrast to NIC, does not undergo biotransformation in hepatocytes (approximately 90–95% of cytisine is excreted unchanged in the urine) [143].

Lactate dehydrogenase is a marker of membrane integrity, commonly used in *in vitro* experiments on isolated heptocytes. The increased lactate dehydrogenase leakage into the medium indicates cell injury. Both cytisine and NIC significantly increased, in a concentration-dependent manner, the release of lactate dehydrogenase into the medium. The toxic effect of cytisine on this parameter was comparable to that of NIC [143].

Hepatotoxicity in vivo. The potential hepatotoxic effect of cytisine was studied *in vivo* in different animal species. In rats, chronic administration of the drug at a dose of 1.35 mg/kg during 90 days caused a 2-fold increase in blood glutamic pyruvic transferase (GPT) concentration, without significant changes in blood glutamic oxaloacetic transferase (GOT) and alkaline phosphatase. Such changes were not observed when cytisine was administered during 45 days to mice (3.3 mg/kg) and 180 days to rats (0.45 and 0.9 mg/kg) or dogs (0.46 mg/kg) [5].

Toxicity for other organs and tissues. Cytisine administered *po* during 5 days in rats at doses of 1 or 5 mg/kg did not show ulcerogenic activity evaluated by macroscopic and histological analysis. Cytisine had more favorable safety profile with respect to the stomach mucosa in comparison with NIC given *po* at equal doses [150].

In experiments assessing the effects of cytisine (Tabex®) on the human kidney epithelial cell line HEK-293T, the drug displayed marginal cytotoxic potential [43]. Changes in transepithelial electrical resistance are indicative of toxic effects. A measurement of transepithelial electrical resistance showed no significant alterations of this parameter upon continuous exposure of HEK-293T monolayers to cytisine. Moreover, a morphological analysis showed that the cell cultures treated with cytisine at the concentrations up to 200 μM did not differ morphologically from the untreated controls. It has been concluded that cytisine is devoid of cytotoxic effects on human kidney cells [43].

In contrast to hepatocytes, cytisine did not exhibit toxic effect on P-diploid human embryonal pulmonary cells, human larynx cancer cell lines HEp-2 or human epitheliod cancer cell lines HeLa [144].

There were no other recorded alterations in the clinical laboratory parameters and histomorphological changes in experiments with the chronic application cytisine to mice (7.6 mg/kg within 30 days) and rats (up to 1.35 mg/kg for 90 days).

Genotoxicity

Cytisine was not genotoxic in the test for cytogenetic aberrations *in vivo* in mice bone marrow cells. When applied orally at doses of 1 mg/kg or 5 mg/kg, it did not induce statistically significant increase in frequency of cells with abnormalities as compared with the control. Only at 10 mg/kg, cytisine induced minimal, non-specific chromosomal abnormalities. In comparison with NIC that caused damage to DNA in epithelial cells [128], cytisine had much lesser clastogenic activity. Higher frequency of chromosomal aberrations was seen even at the lowest dose of NIC (0.1 mg/kg) as compared with the 100-fold higher dose of cytisine (10 mg/kg) [143]. An analysis of the genotoxicity study did not indicate any clastogenic activity of cytisine to be risky for humans [143].

Teratogenicity and embryotoxicity

Potential toxic effects of the alkaloid on reproduction were studied in rats and chicken**.** Administration of cytisine at doses of 3–4 mg/kg to pregnant rats did not result in visible skeletal and visceral abnormalities in fetuses [78]. However, the more complex study by Todorov et al. [144] showed some adverse effects of cytisine on the fetus. The alkaloid at a dose of 9 mg, equal to the maximum human daily therapeutic dose, reduced hen fetal weights by 11%. The doses of 4.5 mg (1/2 the maximum human daily therapeutic dose) and 45 mg (5 times the maximum human daily therapeutic dose) reduced the weights by 4.5 and 52%, respectively. Those percentages of retardation in the weight gain were correlated with the percentages of lethality of hen fetuses which were 8, 32, and 44% for 4.5, 9, and 45 mg/kg, respectively. Experimental data indi-

cate that cytisine shows a tendency to higher toxicity during the first half of embryo development [144].

In addition, cytisine impaired closure of abdominal and thoracic walls that can be possibly explained by the toxic effect of cytisine on the proliferating cells during the early development of the embryos. There is no evidence for skeletal malformations caused by the drug, although curved limbs in fetuses were observed [144]. Furthermore, the histomorphological examination showed dose-dependent dystrophy (from minimal to moderate) of the cells in the heart muscle, liver, stomach, and spleen [144]. There are no studies concerning the potential toxic effects of cytisine in pregnant humans.

Human toxicity

The available human data indicate that cytisine is generally well-tolerated [12, 91, 142, 160]. However, the high doses of the alkaloid can be strongly toxic. It has been known since 1877 [156] that the consumption of the seeds and flowers of *Laburnum anagyroides* or drinking a tea made from the flowers of this plant can cause toxic effects in children or the elderly [35, 51, 53, 97, 123, 155]. Cytisine seems to be the chemical responsible for these responses [53]. The symptoms of cytisine poisoning resemble NIC poisoning symptoms [123].

Although the lethal doses of cytisine in humans were not recorded, there was the report of one fatal case of *Laburnum* seed poisoning in a 50-year-old schizophrenic man who had swallowed 23 pods [123]. The most likely absorbed dose of cytisine was about 50 mg. It has been stated that the lethal dose of cytisine for a dog or cat is about 3–4 mg/kg [27]. Thus, assuming that the man was 75 kg in weight, it would suggest that the lethal dose was about 0.5 mg/kg.

One report identified the toxic effects of cytisine after overdose of Tabex® taken as a suicidal attempt [142]. This report described a case of a 48-year-old woman who took large quantities of the drug. The first time she took 40–50 tablets (1.5 mg/tablet) and experienced initial vomiting, followed by a loss of consciousness and clonic seizures. After admission to an emergency department and recovering consciousness, she experienced muscle spasms, headache, dizziness, weakness, double vision, dysarthric speech, and hypotension. During the hospitalization, she was treated with infusions of sodium chloride and glucose as well as with analeptics, vitamins and phenobarbital. After 5 days, she was discharged from the hospital without any symptoms. Subsequently, she took the second overdose of about 90 tablets of the drug, together with unknown amount of scopolamine and hyoscamine. She fainted again but soon recovered consciousness. No seizures were noted and her symptoms were less severe and after 7 days she was again discharged from the hospital. Noteworthy, no clinical and laboratory features of liver or other internal organ damage were observed.

Cytisine as a medication for nicotine addiction – clinical studies

A literature review identified 12 studies described in 19 papers reporting efficacy and safety of cytisine investigated as a smoking cessation aid. All studies, except for two Russian studies, used cytisine in the marketed form Tabex®, containing 1.5 mg of the compound per tablet. Among all studies, only 3 studies were placebo-controlled [11, 112, 130].

The majority of studies were performed in 1960s and 1970s. Unfortunately, these studies had several design and analysis shortcomings. Their documentation and design would not be considered sufficient to support registration in most European countries because of the lack of appropriate follow-up, and inadequate verification of abstinence criteria. However, the data do suggest that the drug is safe and efficacious at the doses used in the trials. Since 2000, two trials were performed, but only that by Zatonski et al. [160] has been published in scientific literature in English.

Efficacy

During the past 40 years, Tabex® has been used in CEE by millions of smokers trying to stop smoking. More than 4000 subjects have taken Tabex® in efficacy trials.

In the first Bulgarian study reported in 3 papers [140, 141, 142], the treatment groups consisted of a small number of smokers (N = 70 for group I consisting of healthy and psychiatric patients, and N = 17 for group II consisting of psychiatric patients). The dosing regimen of cytisine (Tabex®) followed recommendation of the manufacturer (Tab. 4). The subjects

noticed a marked therapeutic effect occurring as early as the 1st to the 4th day of treatment. After the 20-day treatment course, the successful abstinence rates were 56% and 29% in I and II group as declared by self-report, respectively. However, no longer-term abstinence was assessed and the study lacked adequate definition of abstinence criteria.

The placebo-controlled studies on cytisine (Tabex®) by Paun and Franze [112, 113] adopted a 17-day treatment period with similar dosing regimen. They examined the effect of cytisine in 366 patients *vs*. 239 control patients. Within the cytisine-treated patients, 230 suffered from concomitant chronic bronchitis. The abstinence from smoking was determined by patients' self-report. Among all patients receiving cytisine, 55% were abstinent at the 8th week from the beginning of therapy. At the 26th week, the continuous abstinence rate decreased to 21% patients. In the placebo-controlled part of the study, the abstinence rate in cytisine group (N = 42) was 42% in the 8th week, compared to 33.5% patients in the placebo group (N = 239). However, in contrast to the placebo group, the cytisine-treated patients had additional psychological support (group psychotherapy), which could have affected the results in this group of patients. Similarly to the Bulgarian studies, the cessation was observed most often during the first 5 days of the treatment. Although the treatment groups in the studies by Paun and Franze [112, 113] were larger than those in the Bulgarian studies, the design and documentation of these studies would also not be considered sufficient to support registration of the drug in many European countries.

Other German studies by Benndorf et al. [11–14] and Scharfenberg et al. [129] enrolled 1452 chronic smokers. In the former placebo-controlled, double-blind study (N = 157 in the cytisine-treated group and N = 157 in the placebo group), the abstinence rates were 76% and 70% at the 4th and 16th weeks, respectively. In that study, it was noteworthy that the abstinence rate in the placebo group was significantly higher than usually obtained in other studies and it was 31% at the 16th week [11]. Independently of absolute values of figures in both groups, it should be underlined that smokers treated with Tabex® for 4 weeks had over 2-fold superior abstinence rate compared to subjects treated with placebo. The double-blind study carried out by Scharfenberg et al. [129] compared effectiveness of Tabex® in 607 patients with placebo in the same number of patients. Sixty five and 30.5% of all patients treated with the drug were abstinent at the 4th week and 6th month, respectively, from the beginning of therapy, as compared to 40.5% and 16% in the placebo group, respectively. Two years later, 21% of the patients treated with Tabex® were still non-smokers and this result was significantly higher than that in the placebo group (13%)($p < 0.001$).

Other results examining the efficacy of cytisine in smoking cessation came from a double-blind, placebo-controlled trial carried out by Schmidt [130]. In that study, about 1975 smokers were allocated to receive one of 12 drugs of whom 250 were treated with cytisine (Tabex®). The drug demonstrated an efficacy of 41% after the end of treatment (25-day treatment period at the recommended dosing) *vs*. 31% in placebo group. After 12 weeks this percentage dropped to 27% which was still higher than for placebo (21%), and all the other drugs tested, including Atabakko (caffeine + theobromine), Citotal, Nicobrevin, Nicocortyl, Ni-Perlen, Pempidil, Potassium, *Radix Levistici*, Raucherstop 5-HT, Targophagin, Unilobin, and Viotil. However, because of the reported efficacy rates for several other drugs, it is questionable whether reliable assessments of efficacy were used. No adverse events were observed or reported in the paper.

In one study, buccal films containing cytisine alone (1.5 mg), anabasine alone (1.5 mg) or cytisine (0.75 mg) and anabasine (0.75 mg) were applied for 15 days in three groups consisting of 23, 23, and 16 chronic smokers. Forty seven percent of patients receiving films, including patients with coronary heart disease, hypertension and diabetes mellitus, were reported abstinent at 15 days [105, 106]. Results by group were not indicated but it was stated that the films containing cytisine alone or cytisine in combination with anabasine were more effective than the films containing anabasine alone. The abstinence rate after 6 to 14 months was about 23%. No adverse events were observed/reported in the paper. The data from another study using the same regimen of the treatment, collected after 6 months in 201 smokers (including some psychiatric patients), demonstrated the abstinence rate of 50%, however, the results by groups were not given [95].

A very small but interesting clinical study by Vlaev [152] investigated the possibility of parallel treatment of nicotine addiction and depression. Five patients diagnosed with depression, 3 of them with psychogenic depression and 2 with intermittent depression, were

Tab. 2. Sample characteristics and outcome of an open-label study by Zatonski et al. [160]

Total number of patients attending clinic	438
Number of patients excluded	2
Total of patients enrolled	436
Percentage of (n) males	43.8 (191)
Mean (SD) age (years)	44.4 (13.1)
Percentage of patients (n) smoking more than 10 cigarettes per day	95.0 (414)
Percentage of patients (n) smoking more than 20 cigarettes per day	51.6 (225)
Mean (SD) FTND score (dependence)	6.1 (2.2)
Percentage of patients (n) with post-secondary education	26.8 (117)
Mean (SD) age of starting to smoke regularly	18.9 (4.5)
Percentage of patients (n) having tried to quit before	70.2 (306)
Percentage of patients (n) attending one session	54.1 (236)
Mean (SD) number of visits to clinic	1.7 (0.9)
Number of patients followed up at 12 weeks	342
Number of those followed up who reported taking 1 dose of medication	315
Percentage of patients (n) abstinent at 12 weeks	**27.5 (120/436)**
Percentage of patients (n) reporting gastric disturbance/nausea	10.4 (33/315*)
Percent pf patients (N) stopping medication due to adverse events	15.5 (49/315*)
Number of patients attempted to follow up at 12 months	120
Number of patients followed for up at 12 months	112
Number of patients reporting abstinence	68
Percentage of patients (n) confirmed abstinent at 12 months by CO	**13.8 (60/436)**

*The number reporting having taken medication; CO – exhaled-air carbon monoxide concentration; FTND – Fagerström Test for Nicotine Dependence

treated with ascending doses of cytisine (Tabex®) increased every day to a maximum daily dose of 22.5 mg. As a result, a quick reduction of the depressive symptoms was observed at the end of the 1st week in patients with psychogenic depression, and at the 2nd week in patients with intermittent depression. In addition, the smokers reported a decreased desire to smoke but no detailed data are available. A slight decrease in arterial blood pressure was noted in some patients.

Tab. 3. Adverse effects reported by patients treated with cytisine (Tabex®) in some clinical trials [91, 105, 130, 143, 160]

System organ Signs and symptoms	Frequency (%)
CARDIOVASCULAR	
tachycardia	< 1–14
increase in blood pressure	7
bradycardia	1
cold fingers	1
EYES	
lacrimation	< 1
GASTROINTESTINAL	
dry mouth and throat	35
abdominal pain (mainly upper)	0–20
nausea	1–11.5
constipation	< 1–8
taste changes (mainly bitter taste)	1–4
vomiting	< 1–4
diarrhea	2
flatulence	1
burning tongue	1
heartburn	< 1–2
salivation	< 1
elevation of aminotransferases	< 1
GENERAL	
weakness	7
malaise	1
fatigue	< 1
METABOLISM/NUTRITION	
appetite changes (mainly increased appetite)	47
weight gain	21
NERVOUS	
Neurological	
headache	< 1–17
vertigo	2–4
heaviness in the head	< 1
Psychiatric	
irritability	36
sleep disturbances (insomnia, drowsiness, abnormal dreams, nightmares)	1–21
mood changes	15
anxiety	11
loss of concentration	1–6
dizziness	2
loss of sexual interest	< 1
RESPIRATORY/THORACIC	
dyspnea	< 1
increased expectoration	< 1
SKELETOMUSCULAR	
muscular pain	< 1–10
SKIN/SUBCUTANEOUS TISSUE	
rash	2
increased perspiration	< 1
sagging skin	< 1

In another study on small number of heavy smokers (N = 14), 50% of patients receiving cytisine (Tabex®) were abstinent at 2 weeks after 25-day treatment [91]. Granatowicz [58] reported that 70% of smoking cessation clinic patients treated with cytisine for 27 days (N = 1968) were abstinent at 6-month follow-up. Another study by Marakulin et al. [92] showed very high percentage of abstinent patients at the end of 21-day treatment with Tabex® (70%, N = 388). However, a high percentage of abstinence was also noted in the control group (53%, N = 232). Noteworthy, both groups of patients had 12–14 sessions of autogenic training.

A meta-analysis was recently published based on trials of cytisine for smoking cessation [44]. The meta-analysis of 3 placebo-controlled trials [112, 129, 130] gave a pooled odds ratio of 1.93 (95% confidence interval (CI:1.21–3.06) after up to 8 weeks. For two double blind, placebo-controlled trials with longer follow-up [129, 130], the pooled odds ratio after 3–6 months was 1.83 (CI:1.12–2.99). For one double-blind, placebo-controlled study with follow-up after 2 years [129], the odds ratio was 1.77 (CI:1.29–2.43). For comparison, the odds ratios for different forms of NRT after 12 months was 1.99 (CI:1.5–2.64), and for bupropion 2.06 (CI:1.74–2.4) [45, 64]. The author concluded that cytisine was very probably effective for smoking cessation.

Recently, encouraging results have been seen in an uncontrolled, open-label trial in Poland [160] (Tab. 2). A total of 436 subjects were provided with Tabex® administered as recommended by the manufacturer (Tab. 4) for 25 days with minimal behavioral support. The participants were followed up for up to 12 weeks and those who reported being abstinent by self-report were additionally assessed at 12 months. Their 12-month abstinence, defined by the Russell Standard [154] (up to 5 cigarettes/12 months), was verified by an exhaled carbon monoxide (CO) concentration (<10 ppm). The success rate at 12 weeks was 27.5%. A total of 13.8% of those attending the 12-month smokers' clinic reported being abstinent. This long-term abstinence rate is similar to that noted following NRT [49, 134]. Cytisine reduced craving and withdrawal signs and, for those who smoked while receiving study drug, diminishes smoking satisfaction, e.g. it caused changes in the taste of cigarette in 53% of patients. Although it is impossible to conclude definitively about the efficacy of the drug on the basis of an open-label study, the results of this study confirm previous suggestions that cytisine is useful for smoking cessation. Furthermore, the results of the study supported the argument for randomized controlled study on efficacy of cytisine. A double-blind, placebo-controlled, randomized trial (N = 370/arm) is being planned.

Tab. 4. Recommended therapeutic schedule of cytisine (Tabex®)(Sopharma, Sofia, Bulgaria)

Days	Dose per day (mg)
1–3	9 (6 × 1.5)
4–12	7.5 (5 × 1.5)
13–16	6 (4 × 1.5)
17–20	4.5 (3 × 1.5)
21–25	3 (2 × 1.5)

Tabex® – 1 tabl. contains 1.5 mg of cytisine

Safety and tolerance

A literature review indicates that during over 40 years of cytisine marketing as a smoking cessation aid, no severe adverse reactions have been reported at therapeutic doses [11, 91, 142, 160]. In general, the drug is believed to be well tolerated; however, some adverse effects which can result in discontinuation of the drug were observed. In the available studies, the rate of discontinuation varied from 6% [143] to 15.5% [91, 160].

According to the first study on cytisine safety, 61% of patients treated with the drug had no adverse reactions [141, 142]. Another study reported that in 29% of patients no adverse effects were observed [91]. Adverse effects were usually transient with mild to moderate manifestation. Most often they occurred during the initial phase of therapy and did not result in serious health complications [91, 160].

All adverse events that were reported in currently reviewed research are listed in Table 3. Because safety has been variably and sporadically reported in different historical trials, actual incidence and prevalence of adverse events is difficult to assess. Also, some adverse events may have been due to smoking cessation, and may or may not be attributable to cytisine. It is very likely that clinical manifestations, which were reported as side effects, like irritability, an increase in appetite, weight gain, insomnia or fatigue/malaise, can result from NIC deprivation and were not adverse events of cytisine.

The most common adverse events were gastric distress which occurred in the first days of therapy and usually decreased during the course of treatment. The mechanism of the gastrointestinal adverse effects of cytisine was not fully elucidated. Most likely they result from sympathetic neural stimulation mediated by the drug. The gastrointestinal adverse effects, except for constipation, occurred with similar frequency in patients who stopped and did not stop smoking [160]. Nausea was the most frequent reason of discontinuation of treatment and occurred in about 2/3 of the people who stopped taking the drug. The drop-out rate due to nausea was not higher for cytisine (10%) [160] compared with bupropion (14%) [104]. The reduction of a maximal recommended dose from 9 to 4.5 mg per day and/or administration of H_2-blockers or proton pump inhibitors always brought relief. Granatowicz [58] reported that patients with stomach or duodenum lesions showed no change after taking cytisine. However, since there are no data to support clinical decision, the use of cytisine in patients suffering from an active peptic ulcer disease or gastroesophageal reflux disorder should be very cautious. Also, a particular caution should be taken in patients who had these diseases in the past.

In some cases, a mild elevation of both systolic and diastolic blood pressure as well as a transient mild tachycardia could be observed but usually these symptoms did not cause withdrawal of the treatment. Importantly, the recent study demonstrated that cytisine seems to be safe in hypertensive patients whose blood pressure was sufficiently controlled with the standard antihypertensive drugs [160].

Among all psychiatric symptoms observed after administration of the drug, irritability, insomnia and mood changes were relatively common. Headache was the most frequent neurological adverse effect (up to 17% patients). It occurred with the frequency similar to that observed in patients treated with bupropion (14%) or varenicline (15.5%) [54]. The discontinuation rate due to this symptom was not higher than 3% [160].

Smoking cessation is very often associated with the weight gain. An analysis of the changes in body weight in patients effectively treated with Tabex®, measured before the start of therapy and after 12 months, demonstrated the weight gain of an average of 7 kg in 82% and weight reduction in 17% patients [160]. These results are not consistent with an earlier study which did not find statistically significant changes in body weight of subjects prior to and after the course of treatment [113].

Other adverse reactions listed in Table 3 were relatively rare and did not cause persistent or serious health complications.

Interactions with nicotine and other drugs

Cigarette smoking can affect drug therapy by both pharmacokinetic and pharmacodynamic mechanisms. For example, in animal studies, NIC induced the activity of several cytochrome P450 (CYP) enzymes responsible for the metabolism of a number of drugs [2]. There is a question whether cytisine like NIC can affect the CYP enzymes. So far, any data on this issue have not been identified.

Theoretically, the treatment with cytisine in patients who continue smoking could cause an enhancement of some effects of NIC. There are no reports describing clinically significant reactions that could result from the interaction between NIC and cytisine, and studies investigating this issue are needed.

An influence of cytisine on the action of other drugs has not been established yet. There are only some clinical observations of co-administration of cytisine with other drugs. In one study, cytisine was given to 17 patients treated with neuroleptics due to various psychiatric diseases [142]. Any changes in the efficacy of the treatment of the basic disease were not observed during the course of treatment with cytisine. In addition, 29% of patients stopped smoking but, unfortunately, for a very short period of time. Considering the hepatotoxic potential of some neuroleptics, the authors monitored the liver function in cytisine-treated patients and no abnormal results were found. In the same study, no unfavorable interactions with insulin and antidepressants were observed.

Theoretically, the cardiovascular action of cytisine, i.e. an elevation of blood pressure and heart rate, could be associated with a smaller decrease in blood pressure and heart rate during therapy with β-adrenolytics. Moreover, the antinociceptive activity of cytisine may have clinical consequence by changing the effects of some analgesics. Product information states that cytisine should not be co-applied with tuberculostatic drugs [143] but a reason for this is not given.

Cytisine derivatives and their pharmacology

Cytisine has been a starting point for the studies aimed to search novel compounds of potential therapeutic interest from many years. Some of them were obtained from the natural material, e.g. *N*-methylcytisine, also known as caulophylline. Initially, the structural modifications of cytisine were restricted to improving its respiratory analeptic profile or obtaining local anesthetics [68, 90] but no pharmacological results were published for many obtained compounds. More recently, the studies were mainly aimed at creating derivatives with improved binding affinity and efficacy and minimal side effects. The chemical modifications of cytisine would be expected to increase its lipophilicity, thus improving the ability to pass the blood-brain barrier, to reduce the affinity for ganglionic receptors and to alter its selectivity for different subtypes of central nAChRs.

Cytisine can be structurally modified at the secondary amino groups, conjugated double bonds and carbonyl groups [18]. Substitution of the basic nitrogen atom by a methyl group decreased the activity of cytisine as an nAChRs agonist [10]. *N*-methylcytisine (caulophylline) *in vitro* showed diminished affinity and functional potency at all nicotinic receptor subtypes [135]. The introduction of a nitro group at the 3-position of the pyridone nucleus enhanced the affinity for nAChRs while the introduction of substituents at the basic nitrogen, though reducing to different degrees the affinity, gave rise to compounds with a higher selectivity for central (α4β2*) *versus* ganglionic (α3-containing) receptor subtype [19]. In the study on recombinant human receptors expressed in clonal cell lines and *Xenopus* oocytes, bromination of cytisine at the 5-position of the pyridone ring caused a modest decrease in both affinity and efficacy while iodination caused a decrease in affinity and different effects on efficacy, ranging from a decrease (α7, α4β4* nAChRs) to a marked increase (α4β2* nAChRs) [135]. Bromination of cytisine at the 3-position increased potency in binding assays by about 10-fold at α4β2*, 40-fold at α7 and more than 100-fold at α4β4* nAChRs [63]. With regard to efficacy, the bromo-isosteres of cytisine were more efficacious agonists at α4β4* than at α4β2* nAChRs, mirroring the pattern of efficacy of cytisine [33, 50, 63]. Thus, cytisine and its bromo-isosteres can be useful tools for research purposes to distinguish between different subtypes of nAChRs [151].

Abin-Carriquiry et al. [1] compared the effect of bromination and iodination of cytisine on [^{3}H]DA release from rat striatal preparations. Both 3-bromocytisine and 3-iodocytisine exhibited an increased binding affinity for α4β2* and α7 nAChRs and were more potent than cytisine in evoking [^{3}H]DA release from the rat striatal slices.

Another structural modification of cytisine consists in replacing the pyridone oxygen by a sulfur atom. Thiocytisine, the result of such modification, showed a modest partial agonism at human α4β2* subtype and was inactive at rat α3β4* subtype. Thiocytisine showed an extremely weak, low-efficacy partial agonism at the neuromuscular type of nAChRs [50, 65]. Chellappan et al. [26] found that novel 9-vinyl cytisine derivative had a very similar agonist activity profile to that of cytisine.

Recently, many cytisine derivatives were investigated for biological activities. Some interesting *in vivo* responses were obtained concerning DA antagonism, analgesia, anaphylaxis, an inhibition of stress-induced ulcers, antiinflammmatory and antihypertensive action [18]. For example, 2-methoxyphenylpiperazinylpropyl-cytisine exhibited the strong analgesic activity in the writhing test and formalin test in animals [18]. The same derivative had a strong and long-lasting antihypertensive action in rats. Of interest, the antihypertensive activity was related neither to ganglionic blockade nor α_1- and α_2-adrenoceptor blockade and remains somewhat intriguing. The hypoglycemic activity of an *N*-methyl derivative, realized *via* an increase in insulin release, is also worth mentioning [99]. *In vitro* experiments demonstrated some other biological activities of cytisine analogues, including a positive cardio-inotropic activity, β-antagonism, α_1- and α_2-antagonism, and an inhibition of platelet activating factor-induced platelet aggregation [18].

Despite the synthesis of a number of novel cytisine derivatives, few have succeeded in clinical trials, emphasizing the limitations in translation from screening models to clinical applications. The most thoroughly studied compound, chemically and pharmacologically related to cytisine, is varenicline. Its structure derived from 3-substituted cystinoids [31]. Varenicline, examined at a variety of rat nAChRs expressed in *Xenopus* oocytes, displayed high affinity for α4β2* nAChRs and potent partial agonism at these receptors [31, 33]. Varenicline had lower potency and higher efficacy at

α3β4* receptors and seems to be a weak partial agonist at α3β2* and α6-containing nAChRs, and a full agonist at α7 nAChRs [96]. The pharmacological effects of varenicline on nAChRs are similar to the effects of cytisine. Varenicline mimics the effects of NIC on DA release in the nucleus accumbens when given alone but suppresses this response to a subsequent NIC challenge and reduces NIC self-administration [31, 46]. It should be underlined that its dopaminergic profile is very similar to that of cytisine.

Varenicline has undergone full-scale clinical development and has been recently approved by the U.S. Food and Drug Administration for the treatment of NIC addiction. The good efficacy of the drug in smoking cessation was demonstrated in 6 clinical studies, including 2 comparative trials with bupropion. The CO-confirmed continuous abstinence rate during the last 4 weeks of the therapy with varenicline 1 mg b.i.d. for 12 weeks was 31–45% and it was significantly better than for sustained-release bupropion (29–30%) or placebo (18%) [54, 73, 103, 104]. The continuous abstinence rate from week 9 through 52 across different studies was 19–23% *vs*. 14–16% for bupropion and 4–10% for placebo [54, 73, 145]. The adverse effects were relatively common but did not result in significantly higher discontinuation rate than with placebo [54, 104]. Taken together, the reported results suggest that varenicline represents a promising alternative to agents currently used for the therapy of NIC addiction.

SSR591813 (Sanofi-Synthelabo, France) is another nAChRs ligand chemically and pharmacologically related to cytisine [34]. In *in vitro* and *in vivo* assays, SSR591813 displayed functionally selective partial α4β2* agonist activity. Similarly to cytisine, it behaved as an agonist with lower efficacy than NIC as for its capacity to release DA, and as an antagonist in the presence of NIC. Interestingly, SSR591813 lacked affinity for ganglionic α3β4* nAChRs and lacked cardiovascular effects in animal models of NIC dependence. Like varenicline, SSR591813 may have therapeutic potential in the management of smoking cessation [34] but it needs to be further assessed in clinical trials which ultimately will determine the usefulness of this compound as a smoking cessation aid.

Conclusions

Cytisine, a natural plant alkaloid, used in CEE for 40 years in the clinical management of smoking cessation, has been shown to have pharmacological characteristics similar to NIC. Recent advances in cytisine pharmacological research have elucidated that the drug is a low efficacy partial agonist of α4β2* nAChRs. Cytisine binding to α4β2* receptors can attenuate the consequences of both NIC exposure and its withdrawal. Because of a competitive blockade of α4β2* receptors, cytisine behaves as an antagonist in the presence of NIC; it reduces the DA-releasing and discriminative stimulus effects of NIC. Consequently, it limits the psychogenic reward from NIC obtained through smoking, a key component of tobacco dependence. However, once attached to α4β2* nAChRs, its effect is much weaker than that of NIC. Thus, cytisine would decrease craving and attenuate NIC withdrawal symptoms that often precipitate relapses.

Many clinical studies on cytisine as a smoking cessation aid have suggested that the drug is efficacious and safe; however, these studies do not conform to modern standards in conducting and reporting drug trials, and should be interpreted with caution. Our recent uncontrolled trial conducted in 436 smokers confirmed the previously reported efficacy of cytisine. The 12-month CO verified continuous abstinence rate was similar (13.8%) to that observed following treatment with NRT. In addition to being efficacious, cytisine seems to be well-tolerated. The most frequently reported adverse effects are gastrointestinal in origin. The obvious advantage of the drug is its low cost, which could make it an effective treatment available to millions of smokers.

Since cytisine exhibits a desirable *in vitro* and *in vivo* profile, it should be advanced to randomized controlled trials. Before that, more information on its pharmacokinetics and safety profile in humans for dosages recommended by the manufacturer is required.

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